UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF *CALLICARPA MAINGAYI* K. & G.

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BY

ASIRI, SUMAYYAH MOHAMMED OTHMAN

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2012

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

February 2012

DEDICATION

I dedicate this thesis to:

My father, Mohammed Asiri, who taught me that the best kind of knowledge to have is that which is learned for its own sake.

My mother, Hajar Alaki, who taught me that even the largest task can be accomplished if it is done one step at a time.

My lovely brothers and sisters, whose love and devotion provided me motivation

My friends, whose always have place in my heart

To all those who believe in the richness of learning.

Sumayyah Asiri

Abstract of thesis presented to the Senate of University Putra Malaysia in Fulfillment of the requirement for the degree of Master of Science

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Chairman: Professor Md. Nordin Hj. Lajis, PhD

Institute: Bioscience

Callicarpa maingayi, which is known in Malaysia as "tampang besi" belongs to Lamiaceae family. The plants of the genus *Callicarpa* have been used to treat various ailments, such as malaria, skin cancer, indigestion, dropsy, stomach disorders and rheumatism.

The stem bark of *Callicarpa maingayi* was exhaustively extracted using methanol-water (8:2). The crude extract was successively fractionated using *n*-hexane, chloroform and ethyl acetate. Separation, isolation and purification of compounds were done using solvent-solvent partitioning and chromatographic techniques such as High Performance Liquid chromatography (HPLC), as well as normal phase, reverse phase and Sephadex LH-20 column chromatography.

From the hexane extract, three known compounds were isolated, namely palmitic acid (**91**), tetracosanoic acid (**92**) and a mixture of stigmasterol and *β*-sitosterol (**93**).

The investigation on the chloroform extract led to the isolation of two new naphthoquinone, (+)-callicarpaquinone-A (**97**), callicarpaquinone-B (**96**), along with known avicequinone-C (**95**), which was isolated from the first time from *Callicarpa* species, and 3*β*-hydroxy-lup-20(29)-en-28-oic-acid (**94**).

From the ethyl acetate extract, three known compounds (+)-paulownin (**98**), **(-)**-wodeshiol (**99**), which were isolated for the first time from *Callicarpa* species together with *β*-sitosterol-*β*-D-glucopyranoside (**100**).

The extracts and isolated compounds were screened *in vitro* for anticholinesterase and cytotoxic activities using Ellman's and MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) methods.

The hexane, chloroform and ethyl acetate extracts were inactive against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with IC_{50} values of > 100 μM, but were found to exhibit significant cytotoxicity against liver cancer (HepG2) and breast cancer (MCF-7) cell lines with IC_{50} values ranging from 12.5 to 25.0 μg/mL. The isolated compounds, (+)-callicarpaquinone-A (**97**), callicarpaquinone-B (**96**), avicequinone-C (**95**), **(+)**-paulownin (**98**) and **(-)**-wodeshiol (**99**) were inactive against

AChE with IC_{50} values of > 100 μM. However, they were active against the MCF-7 breast cancer cell line with IC_{50} values of 25.0, 1.9, 2.3, 14.0 and 14.0 μM, respectively.

The structures of the isolated compounds were elucidated using spectroscopic techniques including UV, IR, MS, ^{1}H NMR, ^{13}C NMR, DEPT, HMBC and ^{1}H-^{1}H COSY.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KAJIAN FITOKIMIA DAN BIOAKTIVITI *CALLICARPA MAINGAYI* K. & G.

Oleh

ASIRI, SUMAYYAH MOHAMMED OTHMAN

Februari 2012

Pengerusi: Profesor Md. Nordin Hj. Lajis, PhD

Institut: Biosains

Callicarpa maingayi, dikenali di Malaysia sebagai "tampang Besi" adalah dari keluarga Lamiaceae. Tumbuhan dari genus *Callicarpa* telah digunakan untuk merawat pelbagai penyakit seperti malaria, kanser kulit, senak, dropsi, gangguan perut dan reumatisme

Kulit batang *Callicarpa maingayi* diekstrak menggunakan pelarut metanol air (8:2). Ekstrak mentah diperingkat menggunakan pelarut heksana, kloroform dan etil asetat. Pemisahan, pengasingan dan penulenan sebatian telah dilakukan dengan menggunakan pemeringkatan pelarut dan teknik kromatografi seperti kromatografi cecair prestasi tinggi (HPLC), serta kromatografi fasa biasa, fasa terbalik dan Sephadex LH-20.

Dari ekstrak heksana tiga sebatian yang dikenali telah berjaya dipencilkan iaitu asid palmitik (**91**), asid tetrakosanoik (**92**) dan campuran stigmasterol dan *β*-sitosterol (**93**).

Kajian terhadap ekstrak kloroform telah berjaya mengasingkan dua sebatian naphthokuinon baharu iaitu, callicarpaquinone-A (**97**), callicarpaquinone-B (**96**), bersama-sama dengan avicequinone-C (**95**), yang telah diasingkan buat kali pertama dari spesies *Callicarpa* dan asid 3*β*-hydroxy-lup-20 (29)-en-28oik (**94**).

Dari ekstrak etil asetat 3 sebatian yang dikenali iaitu paulownin (**98**), wodeshiol (**99**), bejaya dipencilkan untuk kali yang pertama dari spesies *Callicarpa* bersama-sama dengan *β*-sitosterol-*β*-D-glucopyranoside (**100**).

Ekstrak mentah dan sebatian terpencil telah diuji untuk aktiviti antikolinesterase dan sitotoksik secara *in vitro* menggunakan kaedah Ellman dan MTT (3-(4,5-dimethylthiazole-2-yl) -2,5-diphenyltetrazolium bromide).

Ekstrak pelarut heksana, kloroform dan etil asetat didapati tidak aktif terhadap activiti antiasetilkolinesterase (AChE) dan antibutirilkolinesterase (BChE) dengan nilai IC_{50} >100 μM tetapi telah menunjukkan aktiviti sitotoksik yang ketara terhadap sel kanser hati (HepG2) dan sel kanser payudara (MCF-7) dengan nilai IC_{50} antara 12.5 kepada 25.0 μg/mL. Sebatian yang berjaya dipencilkan callicarpaquinone-A (**97**), callicarpaquinone-B (**96**), avicequinone-C (**95**), paulownin (**98**) dan wodeshiol (**99**) tidak aktif terhadap aktiviti antiasetilkolinesterase dengan nilai IC_{50} > 100 μM. Tetapi aktif terhadap sel MCF-7 dengan nilai IC_{50} masing-masing sebanyak 25.0, 1.9, 2.3, 14.0 dan 14.0 μM.

Struktur sebatian yang berjaya dipencilkan telah dikenalpasti menggunakan teknik spektroskopi termasuk UV, IR, MS, ^{1}H NMR, ^{13}C NMR, DEPT, HMBC and ^{1}H-^{1}H COSY.

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SUMAYYAH MOHAMMED OTHMAN ASIRI

I certify that an Examination Committee has met on 20/2/212 to conduct the final examination of Asiri, Sumayyah Mohammed on her Master of Science thesis entitled "PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF *CALLICARPA MAINGAYI''* in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Unversiti Putra Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

MOHD ASPOLLAH HJ SUKARI, PhD
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Date:

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement of the degree of Master of Science. The members of the supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

ASIRI, SUMAYYAH MOHAMMED OTHMAN

Date: 20 February 2012

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